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Fate of three insect growth regulators (IGR) insecticides (flufenoxuron, lufenuron and tebufenozide) in grapes following field application and through the wine making process

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Abstract

The dissipation of three insecticide flufenoxuron, lufenuron and tebufenozide residues in grapes after field treatments and during the wine making process was assessed. Residues were determined in grape, must, centrifuged must and wine samples by HPLC-UV after cyclohexane extraction and clean-up on silica phase cartridges. Vines in vineyards with white and red grapes located in Central Greece were sprayed once with commercial formulations of each insecticide at the recommended doses in experiments carried out in 2004 and repeated in 2006. The insecticide residues in grapes showed slow reduction for a period of 42 days after application following first-order kinetics with dissipation rates ranged from 0.011 to 0.018 mg kg\(^{-1}\) d\(^{-1}\). However, at the recommended pre-harvest interval (PHI) residues did not exceed 0.27±0.03 mg kg\(^{-1}\) for flufenoxuron and lufenuron and 0.68±0.07 mg kg\(^{-1}\) for tebufenozide and they were clearly lower than the maximum residue limits (MRLs) set by EU for grape (2 mg kg\(^{-1}\) for flufenoxuron, 1 mg kg\(^{-1}\) for lufenuron and 3 mg kg\(^{-1}\) for tebufenozide). Grape processing into wine caused an almost complete reduction for flufenoxuron and lufenuron as their residues in wine were below the method LOQs (<0.01 mg L\(^{-1}\)), but only a moderate reduction for tebufenozide with concentrations from 0.13 to 0.26 mg L\(^{-1}\) measured in the produced wines. Mean transfer factors for tebufenozide of 0.45 for white ‘Roditis’ and 0.34 for red ‘Cabernet Sauvignon’ were found from grapes into wine for the wines processed without maceration. The wine-making technique (with or without maceration) had the same influence on tebufenozide residues in wine. Of the various clarifying agents studied, charcoal was found to be the only one to be effective in removing tebufenozide residues from wine.

Keywords: IGR insecticides, pesticides, residues, grapes, wine, vinification.
Introduction

The use of insecticides in viticulture is essential for grape protection against pest diseases, and their use is therefore important on grape productivity and on wine quality.

The grape moth (Lobesia botrana) is a wide-spread and harmful pest of grapes. During one season in Greece, this insect has three or four generations, if environmental conditions are favourable. This fact leads to the need for pesticide treatments as near as possible to harvest. The use of these products, particularly when the dose and /or the established pre-harvest time is not observed, can lead to hazardous residues (Fernandez et al. 2005a). It is thus of particular interest to assess the presence of residues of these compounds in grapes and their processed products, such as wine. Among the different chemical classes of new insecticides which have been available the last years to control insects on vine, the benzoylureas (flufenoxuron, lufenuron and other active substances) and the diacylhydrazine tebufenozide are the most promising (Tomlin 2000). These insecticides belong to the family of growth control, so they have the lowest risks for the non-target organisms and their use helps in respecting the environmental balances. Put on the market in the 1990’s to replace the toxic insecticides, they were included in plans for integrated protection.

Since the amount of residues is greatly affected by the pre-harvest interval, high residues could be present in grapes at harvest time, especially when the active substance applied shows high persistence. Consequently, pesticides residues could also be present in wine if the effect of the wine-making technique on residue reduction is poor (Cus et al. 2010, Cabras and Conte 2001). Although data for fungicide residues on grapes and their fate during wine-making are widely available (Garcia-Cazorla and Xirau-Vayreda 1994, Cabras et al. 1997, Cabras et al. 1998, Garcia et al. 1999, Cabras and Conte 2001, Cabras et al. 2001, Fernandez et al. 2005a, Mirlean et al. 2005, Vaquero-Fernandez et
al. 2009, Garau et al. 2009), data for insecticide residues are quite limited (Cabras et al. 1995, Sala et al. 1996, Goodwin and Ahmad 1998, Navaro et al. 1999 and 2001) and even less as far as benzouurea and other insect growth regulator insecticides are concerned (Tsiropoulos et al. 1999). However, the type of wine-making process and the different oenological steps (with or without maceration, clarification, filtration) carried out can influence the reduction of pesticide residues (Fernandez et al. 2005a and b, Oliva et al. 2007, Cabras and Angioni 2000, Ruediger et al. 2004).

This paper aims to contribute to the knowledge of the fate of three common insecticides (flufenoxuron, lufenuron and tebufenozide) residue levels in grapes of two vine cultivars (Roditis and Cabernet Sauvignon) after field applications and during the winemaking process, for which there are very few data concerning the persistence of these active ingredients in grapes and their behaviour during vinification. Furthermore, the influence of different clarifying substances on the removal of residues from wine was studied.

Materials and methods

Chemicals and standards solutions

Analytical standards of flufenoxuron (purity 99.3%) and lufenuron (99.7%) were obtained from Cyanamide (Princeton NJ, USA) and Novartis (Basel, Switzerland), respectively, while tebufenozide (99.9%) was purchased from Dr Ehrenstorfer GmbH. Individual analytical standard stock solutions 1000 mg L⁻¹ for all insecticides were prepared in acetone and stored at −18 °C in glass vials. An intermediate mixture standard solution containing all compounds, at 100 mg L⁻¹ each, was prepared in acetone from the individual stock solutions and stored at −18 °C. Spiking solutions were
prepared by dilution from the intermediate solution and stored at 4°C. Calibration
standard solutions at concentrations 0.05 to 10 mg L\(^{-1}\) (9 solutions) in acetonitrile-water
(1/1, v/v) were prepared from the intermediate solution.

Cyclohexane and dichloromethane were pesticide residue grade, water and
tetrahydrofurane were HPLC-grade and acetonitrile was HPLC-far UV grade. All
solvents were purchased from Labscan (Dublin, Ireland). Silica SPE cartridges (500
mg/3 mL) used for the extracts clean-up were purchased from Isolute (IST Ltd.
International Sorbent Technology, Mid Glamorgan, UK). Bentonite, charcoal,
polyvinylpolypyrrolidone (PVPP), cellulose, potassium caseinate, gelatine and colloidal
silicon dioxide were commercial grade products.

Insecticides formulations Cascade 10 DC (10% flufenoxuron w/v), Match 5 EC (5%
lufenuron w/v) and Mimik 24 SC (24% tebufenozide w/v) were purchased from
commercial market.

Field experiment

The experimental trials were carried out in two vineyards, located at Nea Aghialos,
Magnesia, central Greece. The first vineyard was planted with the white grape cv.
Roditis and the other with the red grape cv. Cabernet Sauvignon. The vines in both
vineyards were spaced 1.20 m on the row and 2.70 m between the rows, and, during the
experimental periods, they received routine horticultural practices.

There were 3 trials in each vineyard for studying the insecticides’ dissipation on
grapes. In each trial an insecticide formulation was sprayed on 27 August 2004 at the
recommended by the manufacturers rates (of 66 mL hL\(^{-1}\) water for Cascade 10 DC
corresponding to 0.066 kg ai ha\(^{-1}\) of flufenoxuron, 100 mL hL\(^{-1}\) water for Match 5 EC
corresponding to 0.05 kg ai ha\(^{-1}\) of lufenuron and 60 mL hL\(^{-1}\) water for Mimik 24 SC
corresponding to 0.144 kg ai ha\(^{-1}\) of tebufenozide), using an automated high pressure mechanically driven applicator (Euro spray Ecology 2000 type 1100 L). Each trial was divided into 4 randomized plots of 15 vines each. Three of them, used as replicates, were treated with the insecticide and the fourth was left untreated to be used as control. Meteorological data were collected by an agrometeorological station, located near the vineyard. During the experimental period (42 days) the average daily air temperature was 20.6 °C, average relative humidity was 64.2% and total rainfall was 18.8 mm.

Field dissipation study of insecticides in grapes was performed during the 2004 period. Grape samples were collected at 0 (3h after application, when the spraying mixture had dried), 2, 4, 7, 14, 21, 28, 35 and 42 days after application (DAA). The samples consisted of randomly collected parts of at least 12 bunches of separate vines and the overall sample weight was 1.5-2 kg with 1 kg the minimum weight recommended in the FAO/WHO guidelines (FAO/WHO, 1986). Grape samples for residues determination were forwarded to the laboratory, blended after removal of the stems, subdivided into 50 g aliquots as analytical replicates and stored in individual bags at -18 °C until extraction.

Application of the insecticides’ formulations in the above vineyard was repeated on 2006 with the same spraying conditions and doses.

**Vinification Process.**

Determination of insecticides was performed on grapes before vinification, in crude must, clear must, racked and filtered wine and clarified wine.

For studying the fate of insecticides residues from vine to wine, samples of grape were processed to produce must and wine. Wine was also produced from grapes collected from the control plot to be used as control sample. Vinification experiments
were performed with grapes collected for each cultivar and each experimental plot at 21 and 30 DAA from the field trials of 2004 and at 21 and 40 DAA from those of 2006 and the vinification was performed at laboratory scale. The collected grape samples (~50 kg) were divided into two equal parts, pressed and stemmed. The one part was allowed to ferment with the skins (vinification with maceration) while the other was separated from the skin and the resulting must was allowed to ferment (vinification without maceration). One hundred mg of sodium metabisulfite and 200 mg of dry yeast were added per L of must in each part. After the completion of alcoholic fermentation (20-25 days) at room temperature, the obtained wine was racked and filtered. A 2x50 g aliquot of the above cloudy must was taken and centrifuged at 4000 rpm for 5 min in order to quantify residues in the clear must.

Clarification tests were carried out on 500 mL of the filtered wine samples. The clarifying agents and the doses employed (usually applied in oenological practice) in separate triplicate samples were as follows: bentonite at 0.4 g L\(^{-1}\), charcoal at 0.2 g L\(^{-1}\), polyvinylpolypyrrolidone (PVPP) at 0.4 g L\(^{-1}\), colloidal silicon dioxide 0.5 g L\(^{-1}\) plus gelatine at 0.025/0.05 g L\(^{-1}\) or potassium caseinate at 0.4 g L\(^{-1}\). After clarification, clear wine and unclarified wine samples were analyzed for insecticide residues.

The samples of grapes before vinification, prepared as described for grape samples in the precedent section, must and clear must, unclarified and clarified wine of sprayed grapes, as well as control wines were conserved at -18 °C until analysis.

*Sample extraction and clean-up procedure*

The investigated insecticides were extracted from the matrices (grapes, must and wine) by a simple and one step extraction using a mixture of cyclohexane-dichloromethane (9/1, v/v) as the extraction solvent described by Likas and Tsiropoulos (2009) and presented briefly below. An aliquot (10 g of previously homogenized grape
sample or 5 mL of wine) was mixed in a centrifuge tube with the extraction solvent mixture (10 mL for grapes or 5 mL for wine). The tubes were well agitated for 30 min in orbital shaker and centrifuged. After centrifugation, an appropriate volume of organic layer (5 mL and 3 mL for grape and wine samples, respectively) was transferred to a pear shape flask, carefully evaporated to dryness with a rotary evaporator at 40°C and the residue was quantitatively transferred with about 1 mL cyclohexane to preconditioned with 10 mL cyclohexane Silica SPE cartridges. After loading of the sample extract, the cartridge was rinsed with 10 mL cyclohexane followed by 3 mL of a cyclohexane-tetrahydrofuran (90/10, v/v) solution. The pesticides were eluted with 2 mL tetrahydrofuran and the eluent was dried under a gentle stream of nitrogen. Residues were re-dissolved in acetonitrile-water (50/50, v/v) solution (in 1 mL for grape and in 0.5 mL for wine) and the resulting solutions were filtered prior to injection into the HPLC system. Unprocessed must and centrifugal must samples were extracted as grape or wine samples, respectively.

**Instrumentation and Chromatographic Conditions**

Chromatographic analysis for the determination of flufenoxuron, lufenuron and tebufenozide was performed with an HP 1100 liquid chromatograph (Hewlett-Packard GmbH, Waldbronn, Germany) equipped with a ternary-delivery system, a variable-wavelength UV detector and an HP ChemStation LC 3D chromatography manager data acquisition and processing system with the ability to obtain UV spectra at selected retention time of chromatograms. The analytical column was a Thermo Hypersil HS C18 column (250 x 2.1 mm I.D with 5 µm particle size) with a guard column and was maintained at 30 °C. The mobile phase was acetonitrile-water delivered at a flow of 0.26 mL min⁻¹ with a gradient composition; from acetonitrile-water (55/45, v/v), held for 5 min, to acetonitrile-water (80/20, v/v) in 10 min, held for 10 min, and finally a
decrease at acetonitrile-water (55/45, v/v) over 10 min to stabilize the HPLC system before starting the next run, giving 35 min as total run time. The injection volume was 20 µL. Before injection, samples were filtered through Titan 2 HPLC nylon membrane filters (17 mm, 0.2 µm pore size). The optimum detection was obtained at 210 nm. Under these chromatographic conditions tebufenozide, flufenoxuron and lufenuron were well separated and their concentrations were determined by the external standard technique by comparing the peak heights in the samples with those found in the calibration solutions.

**Results and discussion**

**Analysis**

The screening method used for pesticides residues determination is simple and suitable for routine analysis. The cyclohexane-dichloromethane solution at 9/1 ratio effectively extracted lufenuron as well as flufenoxuron and tebufenozide. Accuracy data were provided for flufenoxuron, lufenuron and tebufenozide by recovery experiments (n=5) with grape and wine samples at three different levels (0.05-1.00 mg Kg\(^{-1}\) and 0.02-0.25 mg L\(^{-1}\)). The mean recovery percentages for grapes analysis ranged from 88 to 103% for flufenoxuron, 85 to 104% for tebufenozide and 92 to 107% for lufenuron with relative standard deviations (RSDs) <10%. The mean recovery values for wine analysis ranged from 91 to 105% for flufenoxuron, 87 to 99% for tebufenozide and 85 to 106% for lufenuron with relative standard deviations (RSDs) <12%. The limits of quantitation of the method (LOQs), calculated as a signal to noise from untreated grape and wine samples equal to 10. The LOQs for grape analysis was 0.02 mg Kg\(^{-1}\) for flufenoxuron and tebufenozide and 0.05 for lufenuron and for wine analysis 0.005 mg L\(^{-1}\) for flufenoxuron and 0.01 mg L\(^{-1}\) for lufenuron and tebufenozide, depending on the final concentration factor for each extract and the sensitivity of each
compound. Figure 1 shows chromatograms of a mixture standard solution as well as of extracts of grape sample and of the obtained wine sample from the field experiments.

**Field sample analysis and wine-making**

Dissipation of flufenoxuron, lufenuron and tebufenozide residues in “Roditis” and “Cabernet Sauvignon” grape samples from the field experiments is presented in Figures 2, 3 and 4. It should be noticed that the insecticides were applied when grapes had attained their final size and any diluting effect was negligible thereafter. In addition, no residues of the studied insecticides were detected in any of the analysed control grape or wine samples.

The data relating to the residues in grapes, must and wine for the vinification experiments carried out are reported in Tables 1, 2, 3 and 4.

**Flufenoxuron.** After treatment in 2004, flufenoxuron mean concentrations on grapes ranged from 0.33±0.04 to 0.39±0.06 mg kg⁻¹ for “Cabernet” and “Roditis” grapes, respectively. There after residues decreased very slowly with time (Figure 2) showing pseudo-first-order kinetics ($R^2 > 0.944$) with a dissipation rate of 0.011 d⁻¹, and at 42 DAA ~60% of the initial deposited concentration remained in grapes. Similar behaviour with very slow decay rate was also observed for teflubenzuron, an other benzoylurea insecticide, on grapes of Roditis variety (Tsiropoulos et al. 1999). A different fate of flufenoxuron residues was observed in dates where a reduction of 96% of initial flufenoxuron residues was found after 60 days (Kamel et al. 2007).

At 35 DAA, which is the recommended by the manufacturer PHI, flufenoxuron residues on grapes were 0.24±0.03 and 0.26±0.03 mg kg⁻¹ for “Cabernet” and “Roditis”, respectively. For the 2006 experiment, grapes harvested 40 DAA contained
flufenoxuron at 0.27±0.03 and 0.26±0.04 mg kg⁻¹, respectively. Concentration values of flufenoxuron residues measured on all grape subsamples collected few hours after treatment up to near the PHI are clearly below the recently established by EU (31 August 2008) MRL value (2.0 mg kg⁻¹) for flufenoxuron on grapes. Considering our results, we believe that, even after an additional flufenoxuron application 20-30 days before our spraying (i.e. end of July or early August), residues in “Roditis” and “Cabernet” grapes are unlikely to exceed the MRL value even the first day after the second spraying.

Flufenoxuron residues’ partitioning during wine-making process was studied after vinification of grapes collected at 21 and 30 DAA for 2004 (Tables 1 and 2) and at 21 and 40 DAA for 2006 (Tables 3 and 4). The concentration of residues in the must (0.11-0.18 mg kg⁻¹) were significantly lower than in grapes (0.23-0.34 mg kg⁻¹), indicating that flufenoxuron was distributed in the solid parts of grapes (skin) as well as in the liquid part. The mean values of transfer factor of flufenoxuron from grapes to must resulted from all vinification experiments were 0.48 and 0.52, for “Roditis” and “Cabernet” grapes respectively. Centrifugation of the must resulted in major removal of flufenoxuron residues as it was adsorbed by the suspended solids contained in the must and residues in the clear must were 0.02-0.04 mg L⁻¹. At the end of the wine-making process, flufenoxuron residues were not determined in the produced wines (Table 4) with both vinification techniques (with or without maceration) showing the same influence of vinification technique on flufenoxuron residue reduction.

Lufenuron. Lufenuron showed analogous to flufenoxuron persistence on grapes during the field experiments. During 2004, residues of lufenuron on grape samples at 0 DAA ranged from 0.39±0.03 to 0.46±0.09 mg kg⁻¹ for “Cabernet” and “Roditis” grapes,
respectively. Thereafter, lufenuron residues decreased very slowly with time to 0.38±0.03 and 0.39±0.04 mg kg\(^{-1}\) at 7 DAA, and to 0.31±0.04 and 0.28±0.02 mg kg\(^{-1}\) at 21 DAA for “Cabernet” and “Roditis” grapes, respectively. Thereafter, they decreased further to 0.26±0.01 and 0.25±0.03 mg kg\(^{-1}\) (representing 54-66% of the initial residues) at 42 DAA, which is the recommended by the manufacturers PHI for lufenuron. For 2006, grapes harvested at 40 DAA contained lufenuron residues at 0.27±0.03 and 0.24±0.01 mg kg\(^{-1}\) for “Roditis” and “Cabernet” cultivars, respectively. Residues of lufenuron in all our grape subsamples both in 2004 and 2006 were clearly below the recently established by EU (31/8/2008) MRL value (1.0 mg kg\(^{-1}\)) for lufenuron on grapes even few hours after the treatment (at 0 DAA).

Lufenuron dissipation on grapes (Figure 3) showed pseudo-first-order kinetics (\(R^2 > 0.897\)) with low values of reduction rates ranging from 0.011 for “Cabernet” and 0.016 for “Roditis” grapes. Considering our data for flufenoxuron and lufenuron dissipation and the published data for teflubenzuron dissipation in grapes, between the three benzoyleurea insecticides, teflubenzuron seems more persistent than the other two.

The grape samples used to study lufenuron residues partitioning during vinification process were harvested at 21 and 30 DAA for the wine-making process in 2004 and from 21 and 40 DAA for that in 2006. During wine production without maceration and before fermentation, a part of lufenuron residues was removed with the solid part of grapes and the remaining residues in must ranged from 0.09 to 0.16 mg kg\(^{-1}\) with mean values of transfer factor from grapes to must at 0.45 and 0.43, for “Roditis” and “Cabernet” grapes, respectively. After the must centrifugation, lufenuron residues in the clear must were 0.01-0.03 mg L\(^{-1}\) and the calculated transfer factor from must to wine was <0.08, indicating that lufenuron was strongly adsorbed by the suspended solids in the must. Wines obtained at the end of the wine-making process (with and without
maceration) were residue-free (below the method LOQ value of 0.01 mg L⁻¹) and the calculated transfer factor for lufenuron from grape to wine was <0.04 for both “Cabernet” and “Roditis” grapes.

**Tebufenozide.** After treatment, at 0 DAA, mean concentration of tebufenozide residues on grapes were 0.74±0.04 to 0.95±0.14 mg kg⁻¹, for “Cabernet” and “Roditis” grapes, respectively. Tebufenozide residues during the experimental trial dissipated slowly according to pseudo-first-order kinetics (R²= 0.8809-0.8878) with reduction rates ranging from 0.011 for “Cabernet” to 0.018 for “Roditis” grapes (Figure 4). In particular, tebufenozide residues decreased slowly to 0.72±0.05 and 0.68±0.06 mg kg⁻¹ at 7 DAA, then to 0.65±0.06 and 0.59±0.05 mg kg⁻¹ at 14 DAA reaching 0.46±0.05 and 0.40±0.03 mg kg⁻¹ at 42 DAA (representing 62 and 42% of the initially deposited amount on grapes) for “Cabernet” and “Roditis” grapes, respectively. Tebufenozide residues determined in grapes 21 DAA, that is the recommended PHI, ranged from 0.55±0.03 and 0.68±0.07 mg kg⁻¹, values clearly lower than the proposed MRL value for tebufenozide in grapes established by EU (3 mg kg⁻¹) and Codex Alimentarium (2 mg kg⁻¹).

The grape samples used to study tebufenozide partitioning during vinification process were harvested at 21 and 30 DAA for the wine making process in 2004 and at 21 and 40 DAA for that in 2006. Wine prepared with and without maceration from treated grapes of both cultivars contained tebufenozide residues ranging from 0.13 to 0.26 mg L⁻¹ for both vinification processes. The observed concentration of tebufenozide residues in wines produced from grapes collected at or after the recommended PHI exceed the tolerance value established for wine by Switzerland (0.1 mg kg⁻¹) but they are in accordance with the MRL value for tebufenozide in wine (0.3 mg L⁻¹) proposed.
by the International Organisation for Vine and Wine (OIV). The calculated transfer
factors from grape to wine ranged from 0.45 for vinification of white ‘Roditis’ to 0.34
of red ‘Cabernet’ grapes for the wine-making process without maceration and from
0.31 to 0.32, respectively, for the wine-making process with maceration.

During wine production without maceration and before fermentation, residues were
removed with the solid phase of grapes (Tables 1-4) and the remaining residues in must
were 0.17 to 0.33 mg kg\(^{-1}\) resulting in tebufenozide transfer factors from grape to must
0.48 for “Cabernet” and 0.52 for “Roditis”, values almost similar to those observed for
the other two insecticides studied. After the must centrifugation, tebufenozide residues
in the clear must were 0.07-0.12 mg L\(^{-1}\), indicating that tebufenozide was only partially
adsorbed by the suspended solids of the must. The calculated mean values of transfer
factor for tebufenozide from must to wine were 0.73 for “Cabernet” and 0.86 for
“Roditis”, values much higher than those calculated for flufenoxuron and lufenuron
(<0.08). The observed transfer factors of tebufenozide from grapes into must,
centrifuged must, and in wine in all cases indicated a similar pattern of tebufenozide
fate for both cultivars of white ‘Roditis’ and red ‘Cabernet’ grapes.

The clearly different fate of tebufenozide residues relatively to those of
flufenoxuron and lufenuron during the wine-making process is due mainly to their
different distribution capacity between the liquid and solid phase of the produced must
and wine as found from the calculated high tebufenozide transfer factor values from
must to centrifuged must and from must to wine.

Wine clarification experiments performed for wines containing tebufenozide
residues (Figure 5) showed that among the tested clarifying agents, i.e. bentonite,
potassium caseinate, gelatine-silicon dioxide and polyvinylpolypyrrolidone did not
presented significantly decrease the pesticide residue concentration compared with non-
clarified wine. On the other hand, clarification with charcoal significantly reduced (by 95%) tebufenozide residues in wine confirming that it is the most effective between other clarifying agents for reducing various pesticides in wine (Cabras et al. 1995, Tsiropoulos et al. 1999, Ruediger et al. 2004, Fernandez et al. 2005b, Oliva et al. 2007).

Conclusions

All of the ‘insect growth regulator insecticides’ studied showed very slow dissipation in grapes in the field described by pseudo-first-order kinetics and giving dissipation rate values between 0.011 and 0.018 d\(^{-1}\). Their residues’ concentrations at the recommended PHI were clearly below the established MRLs in grape by EU (2 mg kg\(^{-1}\) for flufenoxuron, 1 mg kg\(^{-1}\) for lufenuron and 3 mg kg\(^{-1}\) for tebufenozide), while the measured concentration were below the MRL value even just after application. Therefore, the use of these insecticides should not create limit problems if used following good agricultural practices.

The processing of treated grapes into wine almost eliminated residues for flufenoxuron and lufenuron resulting to residues-free wine, whereas tebufenozide was found in wine at concentrations up to near the 1/10 of the MRL value in grape. The two wine-making techniques employed (with and without maceration) had the same influence on tebufenozide residues in produced wine. Among the clarifying agents used, bentonite, potassium caseinate, gelatine-silicon dioxide and polyvinylpolypyrrolidone did not practically remove residues from wine, while charcoal very effectively eliminated tebufenozide residues.
References


Figures captions

Figure 1. HPLC-UV chromatograms of tebufenozide (peak 1), lufenuron (peak 2) and flufenoxuron (peak 3) standard solution at 0.50 mg/L, of extracts from grape sample collected 30 days post application of tebufenozide, of extract of wine sample produced from the above grapes and of extracts from control grape and wine samples.

Figure 2. Field dissipation of flufenoxuron residues in ‘Roditis’(● —) and ‘Cabernet Sauvignon’ (▲ ---) grapes for 2004 and decline curves as derived from the 1st-order kinetic model. The solid points show mean values of flufenoxuron residues and error bars represent 2xSD of three replicates.

Figure 3. Field dissipation of lufenuron residues in ‘Roditis’(● —) and ‘Cabernet Sauvignon’(▲ ---) grapes for 2004 and decline curves as derived from the 1st-order kinetic model. The solid points show mean values of lufenuron residues and error bars represent 2xSD of three replicates.

Figure 4. Field dissipation of tebufenozide residues in ‘Roditis’(● —) and ‘Cabernet Sauvignon’(▲ ---) grapes for 2004 and decline curves as derived from the 1st-order kinetic model. The solid points show mean values of tebufenozide residues and error bars represent 2xSD of three replicates.

Figure 5. Mean % overall reduction of tebufenozide residues in wines produced from ‘Roditis’ and ‘Cabernet Sauvignon’ grapes after clarification with different clarifying agents.

Tables

Table 1. Flufenoxuron, lufenuron and tebufenozide residues from grapes (mg kg\(^{-1}\)*) to wine (mg L\(^{-1}\)*) for ‘Roditis’ grapes collected at different interval times (days after
application, DAA) for 2004 experiments. Relative standard deviation of three replicates is shown in parenthesis

Table 2. Flufenoxuron, lufenuron and tebufenozide residues from grapes (mg kg\(^{-1}\)) to wine (mg L\(^{-1}\)) for ‘Cabernet Sauvignon’ grapes collected at different interval times (days after application, DAA) for 2004 experiments. Relative standard deviation of three replicates is shown in parenthesis

Table 3. Flufenoxuron, lufenuron and tebufenozide residues from grapes (mg kg\(^{-1}\)) to wine (mg L\(^{-1}\)) for ‘Roditis’ grapes collected at different interval times (days after application, DAA) for 2006 experiments. Relative standard deviation of three replicates is shown in parenthesis

Table 4. Flufenoxuron, lufenuron and tebufenozide residues from grapes (mg kg\(^{-1}\)) to wine (mg L\(^{-1}\)) for ‘Cabernet Sauvignon’ grapes collected at different interval times (days after application, DAA) for 2006 experiments. Relative standard deviation of three replicates is shown in parenthesis
Figure 1

Mix standard solution 0.5 mg/L

Grape sample

Wine sample

Control grape

Control wine
Figure 2

Flufenoxuron

Roditis: $y = 0.385e^{-0.0108x}$
$R^2 = 0.9623$

Cabernet: $y = 0.3294e^{-0.0108x}$
$R^2 = 0.9439$
Figure 3
Figure 4

Tebufenoizde

\[ y = 0.7487e^{-0.0101x} \quad R^2 = 0.8809 \]

\[ y = 0.8529e^{-0.0183x} \quad R^2 = 0.8878 \]
Figure 5
Table 1. Flufenoxuron, lufenuron and tebufenozide residues from grapes (mg kg\(^{-1}\)*) to wine (mg L\(^{-1}\)**) for ‘Roditis’ grapes collected at different interval times (days after application, DAA) for 2004 experiments. Relative standard deviation of three replicates is shown in parenthesis.

<table>
<thead>
<tr>
<th></th>
<th>Flufenoxuron</th>
<th>Lufenuron</th>
<th>Tebufenozide</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DAA</td>
<td>21</td>
<td>30</td>
</tr>
<tr>
<td>Grapes *</td>
<td></td>
<td>0.31 (14)</td>
<td>0.28 (11)</td>
</tr>
<tr>
<td>Must *</td>
<td></td>
<td>0.14 (10)</td>
<td>0.13 (9)</td>
</tr>
<tr>
<td>Centrifuged Must **</td>
<td></td>
<td>0.03 (12)</td>
<td>0.02 (13)</td>
</tr>
<tr>
<td>Wine with maceration **</td>
<td>&lt;LOQ</td>
<td>&lt;LOQ</td>
<td>&lt;LOQ</td>
</tr>
<tr>
<td>Wine without maceration **</td>
<td>&lt;LOQ</td>
<td>&lt;LOQ</td>
<td>&lt;LOQ</td>
</tr>
</tbody>
</table>
Table 2. Flufenoxuron, lufenuron and tebufenozide residues from grapes (mg kg⁻¹*) to wine (mg L⁻¹**) for ‘Cabernet’ grapes collected at different interval times (days after application, DAA) for 2004 experiments. Relative standard deviation of three replicates is shown in parenthesis.

<table>
<thead>
<tr>
<th>DAA</th>
<th>Flufenoxuron 21</th>
<th>Flufenoxuron 30</th>
<th>Lufenuron 21</th>
<th>Lufenuron 30</th>
<th>Tebufenozide 21</th>
<th>Tebufenozide 30</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grapes *</td>
<td>0.24 (10)</td>
<td>0.23 (12)</td>
<td>0.31 (11)</td>
<td>0.27 (8)</td>
<td>0.68 (10)</td>
<td>0.56 (12)</td>
</tr>
<tr>
<td>Must *</td>
<td>0.13 (14)</td>
<td>0.11 (15)</td>
<td>0.16 (9)</td>
<td>0.12 (15)</td>
<td>0.27 (12)</td>
<td>0.33 (18)</td>
</tr>
<tr>
<td>Centrifuged Must **</td>
<td>0.03 (9)</td>
<td>0.02 (11)</td>
<td>0.01</td>
<td>0.02 (17)</td>
<td>0.08 (7)</td>
<td>0.11 (10)</td>
</tr>
<tr>
<td>Wine with maceration **</td>
<td>&lt;LOQ</td>
<td>&lt;LOQ</td>
<td>&lt;LOQ</td>
<td>&lt;LOQ</td>
<td>0.21 (12)</td>
<td>0.17 (9)</td>
</tr>
<tr>
<td>Wine without maceration **</td>
<td>&lt;LOQ</td>
<td>&lt;LOQ</td>
<td>&lt;LOQ</td>
<td>&lt;LOQ</td>
<td>0.23 (9)</td>
<td>0.19 (11)</td>
</tr>
</tbody>
</table>
### Table 3

Flufenoxuron, lufenuron and tebufenozide residues from grapes (mg kg\(^{-1}\)*) to wine (mg L\(^{-1}\)**) for ‘Roditis’ grapes collected at different interval times (days after application, DAA) for 2006 experiments. Relative standard deviation of three replicates is shown in parenthesis.

<table>
<thead>
<tr>
<th>DAA</th>
<th>Flufenoxuron</th>
<th>Lufenuron</th>
<th>Tebufenozide</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Grapes *</td>
<td>Must *</td>
<td>Centrifuged Must **</td>
</tr>
<tr>
<td></td>
<td>0.32 (9)</td>
<td>0.17 (11)</td>
<td>0.03 (8)</td>
</tr>
<tr>
<td></td>
<td>0.26 (14)</td>
<td>0.12 (10)</td>
<td>0.02 (7)</td>
</tr>
<tr>
<td></td>
<td>0.33 (8)</td>
<td>0.14 (11)</td>
<td>0.02 (12)</td>
</tr>
<tr>
<td></td>
<td>0.27 (10)</td>
<td>0.12 (5)</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>0.69 (12)</td>
<td>0.30 (9)</td>
<td>0.09 (12)</td>
</tr>
<tr>
<td></td>
<td>0.35 (9)</td>
<td>0.17 (10)</td>
<td>0.07 (7)</td>
</tr>
<tr>
<td></td>
<td>&lt;LOQ</td>
<td>&lt;LOQ</td>
<td>&lt;LOQ</td>
</tr>
</tbody>
</table>
Table 4. Flufenoxuron, lufenuron and tebufenozide residues from grapes (mg kg\(^{-1}\)*) to wine (mg L\(^{-1}\)**) for ‘Cabernet Sauvignon’ grapes collected at different interval times (days after application, DAA) for 2006 experiments. Relative standard deviation of three replicates is shown in parenthesis.

<table>
<thead>
<tr>
<th></th>
<th>Flufenoxuron</th>
<th>Lufenuron</th>
<th>Tebufenozide</th>
</tr>
</thead>
<tbody>
<tr>
<td>DAA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>0.34 (9)</td>
<td>0.29 (8)</td>
<td>0.73 (12)</td>
</tr>
<tr>
<td>40</td>
<td>0.27 (11)</td>
<td>0.24 (6)</td>
<td>0.39 (9)</td>
</tr>
<tr>
<td>Grapes *</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>0.18 (8)</td>
<td>0.11 (10)</td>
<td>0.32 (11)</td>
</tr>
<tr>
<td>40</td>
<td>0.14 (7)</td>
<td>0.09 (9)</td>
<td>0.19 (7)</td>
</tr>
<tr>
<td>Must *</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.04 (12)</td>
<td></td>
<td>0.01</td>
<td>0.07 (5)</td>
</tr>
<tr>
<td>Centrifuged Must **</td>
<td></td>
<td>0.02 (9)</td>
<td>0.09 (10)</td>
</tr>
<tr>
<td>Wine with maceration **</td>
<td>&lt;LOQ</td>
<td>&lt;LOQ</td>
<td>0.25 (8)</td>
</tr>
<tr>
<td>Wine without maceration **</td>
<td>&lt;LOQ</td>
<td>&lt;LOQ</td>
<td>0.23 (5)</td>
</tr>
</tbody>
</table>